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Biological and Genomic Differences of ERG Oncoprotein-Stratified Prostate Cancers from African and Caucasian Americans

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT There is striking disparity in prostate cancer (CaP) incidence and mortality for AfricanAmerican (AA) men as compared to Caucasian Americans (CA). Biological basis for this disparity has not been established. Oncogenic activation of ERG resulting from prevalent gene fusions is present in two thirds of CaP patients in Western countries.					
15. SUBJECT TERMS- Prostate Cancer, health disparity, ERG oncogene, molecular stratification, germline variants (SNPs), admixture mapping, European and African ancestry, somatic mutations, aggressive cancer, nomograms					
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1. INTRODUCTION:

It is **hypothesized** that ERG gene fusion status of prostate tumors reflects the underlying biological or genetic differences of prostate cancer (CaP) incidence and/or progression between African American (AA) and Caucasian American (CA) patients. The **objective** of this proposal is to determine associations and racial differences of key clinico-pathologic features and SNPs for both ERG positive and ERG negative CaP. It is anticipated that molecular determinants of aggressive CaP in AA men include somatic mutations (TMPRSS2-ERG) and germline variants (SNPs).

The objective will be achieved by the following specific aims:

Aim 1: ERG-typing based molecular stratification of AA CaP patients. The goal of this aim is to establish our novel findings of lower ERG frequency in AA than in CA CaP, especially in tumors with high Gleason grade. ERG oncoprotein expression will be evaluated in whole-mounted prostates of 400 AA compared to 200 CA CaP patients.

Aim 2: Determine germline genetic determinants of the somatically acquired TMPRSS2-ERG fusion in AA men. We propose to use admixture mapping as it is particularly well suited for traits that present a sizeable difference in prevalence rates, such as the TMPRSS2-ERG fusion. Ancestry at each point in the genome in AA men will be estimated. Regions in AA genomes that are enriched for European ancestry in cases with the fusion compared to cases without the fusion will be captured. A total of 400 AA individuals with CaP will be genotyped and analyzed by HAPMIX program to infer local ancestry.

Aim 3: Define CaP driver mutations in ERG negative high grade tumors. Recently identified CaP driver mutations present in ERG negative CA-CaP will be directly assayed for, including SPOP mutation and SPINK1 overexpression. The PTEN/AKT pathway, which is often associated with aggressive CaP, will also be tested in this cohort by PTEN expression assay. Finally, we propose that the incorporation of ERG-typing, somatic mutations/markers in ERG-negative CaP, and ERG-type associated SNPs, will complement traditional pathological and clinical feature-based nomograms and lead to improved identification of aggressive CaP in AA patients.

Scope: This study will define the underlying biology and genetics of the ERG positive and ERG negative prostate tumors in AA and CA patients with special focus on the features of ERG negative aggressive CaP in AA patients.

2. KEYWORDS:

Prostate cancer, health disparity, ERG oncogene, molecular stratification, germline variants (SNPs), admixture mapping, European and African ancestry, somatic mutations, aggressive cancer, nomograms

3. ACCOMPLISHMENTS:

- **What were the major goals of the project (as stated in the SOW)?**

Major Task 1: ERG-typing based molecular stratification of AA CaP patients

Subtasks: ERG oncoprotein expression in 600 whole-mounted prostates from 400 AA compared to 200 CA CaP patients will be evaluated. The specimen cohorts will be identified from the CPDR tissue bank archive with up to 15 years follow-up time, excluding neo-adjuvant treated patients.

- IRB protocol approval
- Selection of AA and CA patient cohorts
- Identification of the archived whole mounted prostate specimens from the CPDR Tissue Bank
- Selection of the best representative blocks (includes index tumor and other tumor foci)
- Sectioning the blocks (10 unstained sections and an H&E stained section from each block)

- IHC with CPDR ERG MAb (clone 9FY)
- ERG IHC reading by pathologist
- Statistical analysis of the data
- Data interpretation, summary of Task 1 for manuscript

Timeline: Months 1-16

Major Task 2: Define germline genetic determinants of the somatically acquired TMPRSS2-ERG fusion in AA men

Subtasks: We propose to use admixture mapping to estimate ancestry at each point in the genome in AA men. Regions in AA genomes that are enriched for European ancestry in cases with the TMPRSS2-ERG fusion, compared to cases without the fusion, will be captured. A total of 400 AA individuals will be genotyped and analyzed by HAPMIX program to infer local ancestry.

- Blood genomic DNA specimens from the 400 AA CaP patients will be prepared (CPDR site).
- The DNA specimens from the 400 individuals will be genotyped on the Illumina Golden Gate genotyping platform.
- The HAPMIX program will be used for the analysis to infer local ancestry.
- Two statistical tests that are both implemented in HAPMIX will be utilized. The case-only admixture association (ADM) and sum of case-control SNP association and case-only admixture association (SUM) statistics will ensure the appropriate null distribution.
- Data interpretation, summary of Task 2 for manuscript

Timeline: Months 6-24

Milestone #1: Submit manuscript on Tasks 1 and 2

Major Task 3: Define CaP driver mutations in ERG negative high grade tumors

Subtasks: Somatic changes including expression (SPINK1), deletion (PTEN) and point mutations (SPOP) will be determined in the ERG negative subset of the 600-patient cohort. ERG-type associated SNPs and somatic markers will be assessed for improvement of prognostic nomogram.

- Unstained sections from the 600 blocks (400 from AA and 200 from CA patients) from Task 1a will be utilized in Task 3.
- Marker genes of pathways in aggressive CaP with ERG negative status will be tested in this cohort.
 - a. SPINK1 overexpression will be assayed for by IHC following optimized procedure (Tomlins et al, 2008)
 - b. PTEN expression will be determined by IHC assay (Lotan et al, 2011; Chaux et al, 2012)
 - c. The stained slides will be read by our GU pathologist, and will also be quantified by specialized image analysis software (Definiens, Parsippany, NJ)
- SPOP mutations reported in CaP with ERG negative status will also be tested (Barbieri et al, 2012)
 - a. Tumor areas from the whole mounted prostate tissue sections will be dissected with the ArcturusXT laser capture microdissection (LCM) Instrument (Life Technologies)
 - b. DNA will be purification from the microdissected tissue and amplified by Whole Genome Amplification kit (WGA4), as suggested by the manufacturer for the single-cell approach (Sigma-Aldrich)
 - c. Standard PCR will be used for targeted enrichment of SPOP exon 6 and exon 7 followed by sequencing.

- d. Statistical analysis of the summarized data with clinical and pathological parameters focusing on disease progression will be performed by the bio-statistician and the epidemiologist at CPDR
- Finally, ERG-typing, somatic mutations/markers in ERG-negative CaP, and ERG-type associated SNPs will be incorporated into the best available widely used postoperative prognostic nomogram to complement traditional pathological and clinical feature-based nomograms with the goal to improve identification of aggressive CaP in AA patients
 - a. All SNPs and gene expression marker candidates (individually and in combinations) will be tested for their significance in multivariate statistical models (Cox analysis) in which the potential markers will be added to standard clinical variables
 - b. The postoperative prognostic nomogram with and without a marker candidate will be assessed for improvement of the concordance index.

Timeline: Months 16-36

Milestone #2: Submit manuscript on Task 3

- **What was accomplished under these goals?**

Major Task 1 has been completed as scheduled in the Statement of Work. In this first year of the proposed project the originally stated Aim1 was performed:

After obtaining IRB approval for the project, the cohort of **600 CaP patients** (400 AA and 200 CA) have been selected from the CPDR Biospecimen Banks as consecutive cases treated by radical prostatectomy (RP) at the Walter Reed National Military Medical Center (WRNMMC, formerly WRAMC) between 1997 and 2007. Patients with less than 5 years follow-up time or neo-adjuvant treatment were excluded. **Archived whole mounted prostate specimens** have been identified from the CPDR Tissue Bank. The best representative blocks (including the index tumor and other tumor foci) were processed by cutting consecutive 4 micron sections, the first and last **stained by H&E for pathological evaluation**. In this aim one section per each patient have been **stained by IHC using the CPDR ERG MAb (clone 9FY) following our optimized procedure** (6). The 600 whole mounted prostate slides stained by ERG IHC were be read by our GU pathologist. Statistical analysis of the summarized data was performed by the bio-statistician and the epidemiologist at CPDR. The expected difference in ERG frequency between AA (30%) and CA (60%) was apparent. Based on preliminary data from our laboratory (5) a focused analysis on the high grade cases was performed to explore increased ethnic differences:

Evaluations of the ERG alterations at the genomic, transcript and protein levels have continued to suggest lower frequencies of ERG in AA CaP in comparison to CA CaP (1-5). Almost complete concordance between the detection of ERG gene fusions by fluorescent in situ hybridization (FISH) and ERG protein detection by immunohistochemistry, has greatly accelerated the evaluations of ERG protein as the surrogate of this common CaP genome alterations in pathologic specimens (6-9). Studies from our and other groups indicate that overall frequency of ERG alterations in CaP vary greatly among different ethnicities: highest in CA, intermediate in AA and lowest in the Asians (10, 11). Our recent evaluations of representative whole-mount prostate sections from a matched cohort of 91 CA and 91 AA men showed a significant difference ($p < 0.0001$) in the prevalence of the ERG oncoprotein in index tumors of CA (63%) and AA (29%) men (5). Our preliminary data also suggested that the majority of higher grade tumors in AA patients may be ERG negative (5). **This study now focuses on comparative evaluations of ERG in higher grade prostate tumors of CA and AA patients.**

This adequately powered study (126 patients: 63 CA, 63 AA) used matched cohorts of CA and AA specimens (**Table 1**). The index tumor and all other tumors were classified as ERG-positive (any number of tumor cells positive) or negative (all tumor cells negative). **Figure 1** provides representative examples. A striking finding was that ERG was significantly (3 times) more likely to be present in the higher grade index tumors of CA men compared to AA men (31 of 63 vs. 10 of 63 patients, $p<0.0001$) (**Table 2**). Thus, although ERG may be the most common oncogenic alteration in CA men, it does not seem to be the case in AA men, especially not in higher grade CaP. The biological basis underlying this observation remains to be developed, these results nonetheless support association of ERG negative status with more aggressive disease in AA men, underlining that in these patients ERG may not be the primary driver of CaP.

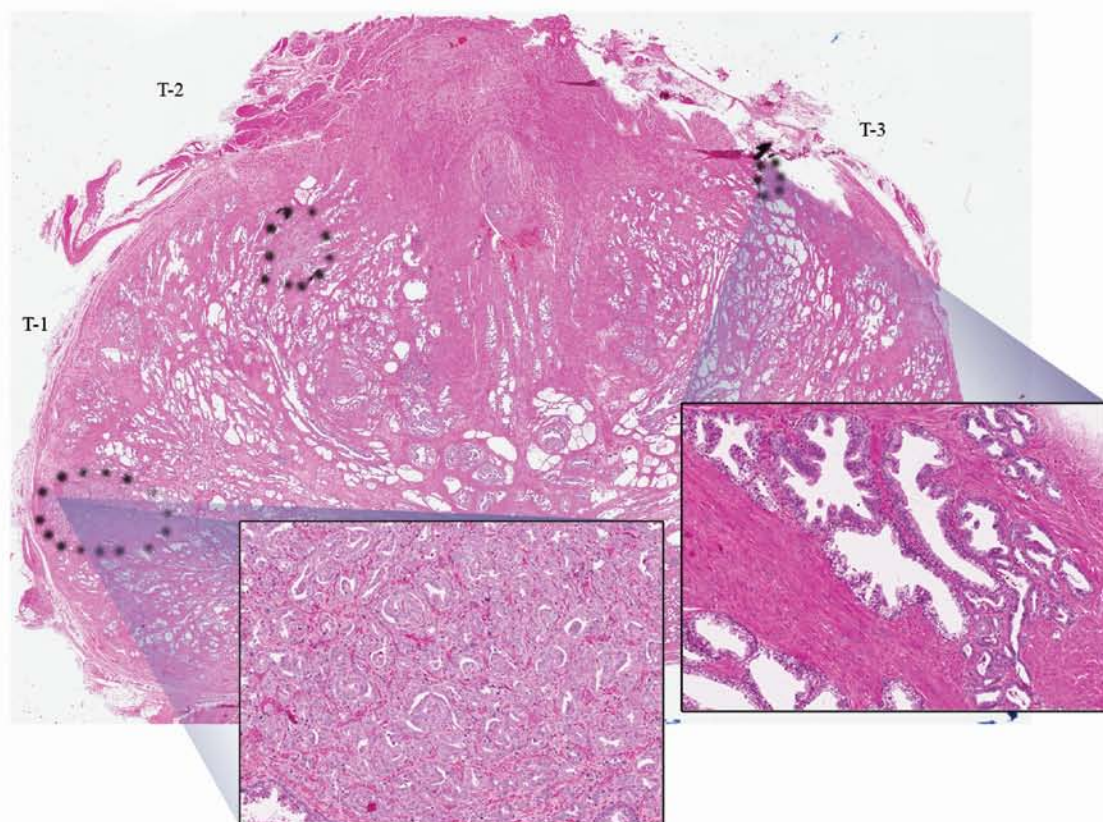
While there is a general agreement that ERG is a highly prevalent and early oncogenic alteration in CaP and it defines a large sub-type of prostate tumors, it is also important to recognize that there are significant proportions of ERG negative prostate tumors for which a common driver gene alteration is not known. Emerging data from this and other studies underscore the higher prevalence of the ERG negative sub-type of CaP in AA and Asian men (10, 11). The higher frequency of high grade ERG negative tumors in AA men likely reflects the presence of distinct genomic alterations associating with initiation as well as progression of this sub-type of CaP.

In summary, this study provides striking observations on the predominance of ERG negative high grade CaP in AA men. ERG expression was significantly (3 times) more likely to be present in the higher grade index tumors of CA men compared to AA men (31 of 63 vs. 10 of 63 patients, $p<0.0001$). The biological implications of these observations are far reaching especially in delineating biological typing and future treatment of CaP tumors in men of different ethnicities.

Variable	All	AA	CA	P value
Age at RP (yr)				0.5887
Mean (SD)	60.4 (7.1)	60.1 (7.2)	60.8 (7.1)	
PSA at diagnosis (ng/mL)				0.2718
Median (range)	6.7 (0.9-5065)	6.9 (1-5065)	6.5 (0.9-23.4)	
Pathological T stage				0.2008
pT2	49 (38.9)	28 (44.4)	21 (33.3)	
pT3 or above	77 (61.1)	35 (55.6)	42 (66.7)	
Gleason sum				0.8538
4+3	47 (37.3)	24 (38.1)	23 (36.5)	
8 to 10	79 (62.7)	39 (61.9)	40 (63.5)	
ECE				0.6855
Negative	49 (43.0)	26 (44.8)	23 (41.1)	
Positive	65 (57.0)	32 (55.2)	33 (58.9)	
SV				0.2496
Negative	91 (72.8)	48 (77.4)	43 (68.2)	
Positive	34 (27.2)	14 (22.6)	20 (31.8)	
Margin status				0.3230
Negative	83 (69.2)	44 (73.3)	39 (65.0)	
Positive	37 (30.8)	16 (26.7)	21 (35.0)	

Table 1. Clinico-pathologic characteristics of all patients and breakdown across racial cohorts

A



B

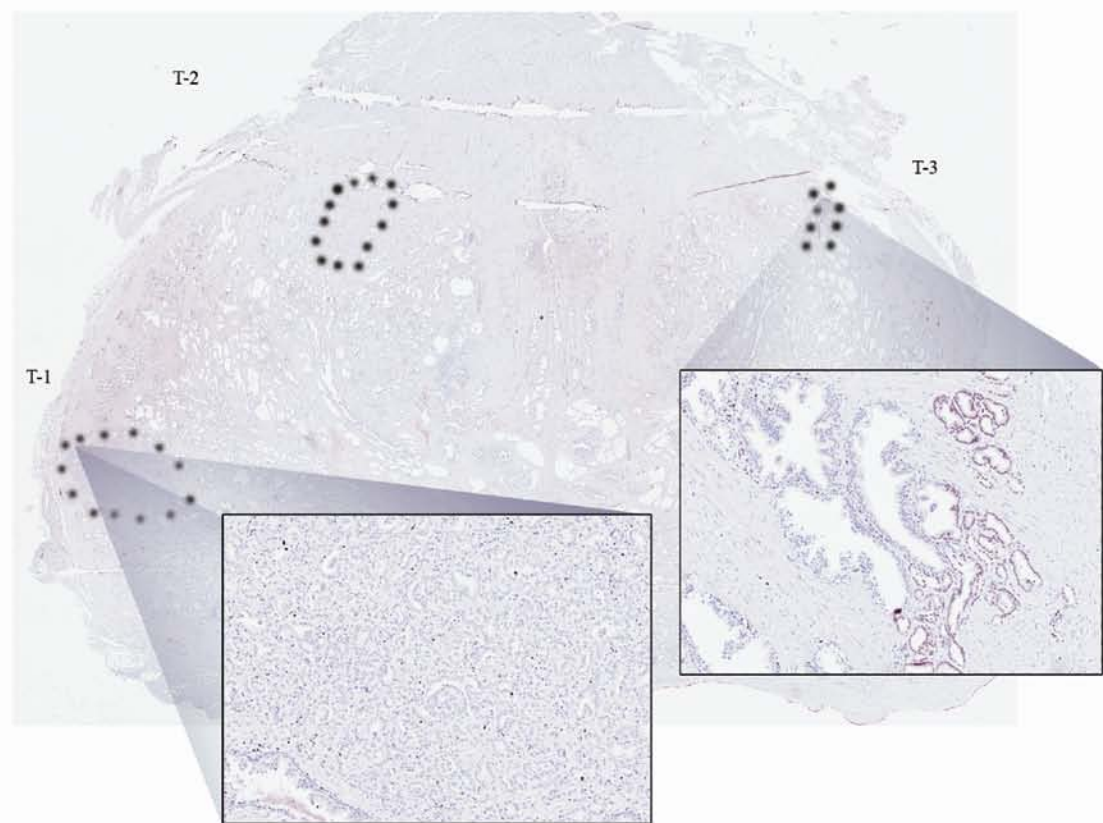


Figure 1. Representative images of whole mount sections analyzed by H&E stain as well as ERG IHC, with view fields enlarged. (A) H&E stain with tumor foci denoted by dotted outline. The higher power insert from T1 (index tumor) contains poorly differentiated, Gleason 4 disease. The T3 (tertiary tumor) insert on the right is well differentiated, Gleason 3. (B) Analogous section with ERG IHC staining, in which the nuclear stain for ERG is negative in T1 and focally positive in T3.

	Total	CA	AA	P-value
ERG+ high grade	33% (41/126)	49% (31/63)	16% (10/63)	<0.0001
ERG+ low grade*	52% (35/67)	69% (24/35)	34% (11/32)	0.0051
P-value		0.0642	0.0400	

*Data obtained from Rosen *et al* 2012

Table 2. Prevalence of ERG positivity across race in high grade (Gleason 8-10 and 4+3) index tumors (upper lane, this study) and in low grade (Gleason 6) index tumors (lower lane)

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2. Hu Y, Dobi A, Sreenath T, et al: Delineation of TMPRSS2-ERG splice variants in prostate cancer. *Clin Cancer Res* 14: 4719-4725, 2008.
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4. Magi-Galluzzi C, Tsusuki T, Elson P, et al: TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients. *Prostate* 71: 489-497, 2011.
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7. Park K, Tomlins SA, Mudaliar KM, et al: Antibody-based detection of ERG rearrangement-positive prostate cancer. *Neoplasia* 12: 590-598, 2010.
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9. Rosen P, Sesterhenn IA, Brassell SA, et al: Clinical potential of the ERG oncoprotein in prostate cancer. *Nature Reviews Urology* 9: 131-137, 2012.
10. Martin DN, Starks AM, and Ambs S: Biological determinants of health disparities in prostate cancer. *Curr Opin Oncol* 25: 235-244, 2013.
11. Farrell J, Petrovics G, McLeod DG, and Srivastava S: Genetic and molecular differences in prostate carcinogenesis between African American and Caucasian American men. *Int J Mol Sci* 14: 15510-15531, 2013.

- **What opportunities for training and professional development has the project provided?**

Nothing to report

- **How were the results disseminated to communities of interest?**

Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

Focus on completion of **Major Task 2:** Define germline genetic determinants of the somatically acquired TMPRSS2-ERG fusion in AA men

Subtasks: We propose to use admixture mapping to estimate ancestry at each point in the genome in AA men. Regions in AA genomes that are enriched for European ancestry in cases with the TMPRSS2-ERG fusion, compared to cases without the fusion, will be captured. A total of 400 AA individuals will be genotyped and analyzed by HAPMIX program to infer local ancestry.

- Blood genomic DNA specimens from the 400 AA CaP patients will be prepared (CPDR site).
- The DNA specimens from the 400 individuals will be genotyped on the Illumina Golden Gate genotyping platform.
- The HAPMIX program will be used for the analysis to infer local ancestry.
- Two statistical tests that are both implemented in HAPMIX will be utilized. The case-only admixture association (ADM) and sum of case-control SNP association and case-only admixture association (SUM) statistics will ensure the appropriate null distribution.
- Data interpretation, summary of Task 2 for manuscript

Major Task 3 will also be started focusing on the SPINK1 and PTEN analysis.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

Our findings on the predominance of ERG negative high grade prostate cancer in AA men, compared to CA men, impact the area of **genetic aspects of racial disparity in prostate cancer**. Our unique patient cohort, treated by radical prostatectomy at the Walter Reed National Military Medical Center, is within the **equal access DOD healthcare beneficiary system**. In this system socio-economic factors influencing disparity are less pronounced leaving genetic factors easier to pinpoint. The finding that the expression of ERG, a major early oncogene in prostate cancer, was significantly (3 times) more likely to be present in the higher grade index tumors of CA men compared to AA men in a tightly matched cohort of 126 patients (Farrell *et al*, 2014) clearly supports that besides socio-economic factors the **somatic genetic events in the prostate tissue may also be different between ethnic groups potentially impacting racial disparity of the disease**.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

- **Changes that had a significant impact on expenditures**

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

- **Significant changes in use or care of human subjects**

Nothing to report

- **Significant changes in use or care of vertebrate animals**

Nothing to report

- **Significant changes in use of biohazards and/or select agents**

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
Journal publications.

Farrell J, Young D, Chen Y, Cullen J, Rosner IL, Kagan J, Srivastava S, McLeod DG, Sesterhenn IA, Srivastava S, Petrovics G. Predominance of ERG-negative high-grade prostate cancers in African American men. Mol Clin Oncol. 2014 Nov;2(6):982-986.

- **Books or other non-periodical, one-time publications.**

Nothing to report

- **Other publications, conference papers, and presentations.**

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

- **What individuals have worked on the project?**

Gyorgy Petrovics, PI, (2.4 person months) The PI provides the overall organization for the execution of the specific aims. He coordinated the selection and processing of patient tissue specimens in close collaboration with Dr. Sesterhenn, and coordinates with Dr. Freedman the genotyping efforts and with Dr. Cullen the data analyses. He closely supervises the postdoctoral fellow's (Dr. Indu Kohaar), Ms. Young's and Ms. Ravindranath's experimental work related to this proposal.

Matthew Freedman, Qualified Collaborator, (1.2 person months) Oversees and organizes the genotyping operations in close collaboration with the PI.

Denise Young, Histology Technologist, (1.4 person months) Manages, prepares, and maintains the histologic preparations using state-of-the-art histopathology and molecular pathology procedures pertinent to this proposal under the directions of the PI and Dr. Sesterhenn. Ms. Young performs histological procedures and analytical procedures including tissue sectioning, staining and mounting specimens on slides, immunohistochemistry (IHC) staining of whole mounted prostate sections and optimizing procedures to assure the successful outcome of the proposed experiments.

Indu Kohaar, Postdoctoral Fellow, (6.0 person months) Dr. Kohaar has experience in, and performs, mutation and SNP analysis, IHC assays, QRT-PCR experiments and bDNA analysis with selected markers for this proposal under the direction of the PI.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organizations were involved as partners?**

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS:

Nothing to report

9. APPENDICES:

A copy of a journal article is attached supplementing and supporting the text:

Farrell J, Young D, Chen Y, Cullen J, Rosner IL, Kagan J, Srivastava S, McLeod DG, Sesterhenn IA, Srivastava S, Petrovics G. Predominance of ERG-negative high-grade prostate cancers in African American men. *Mol Clin Oncol*. 2014 Nov;2(6):982-986.

Predominance of ERG-negative high-grade prostate cancers in African American men

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Abstract. Erythroblast transformation-specific-related gene (ERG) fusions, the most common and validated prostate cancer (CaP) genome alteration, result in alterations in the expression of the ERG oncoprotein. Significantly lower frequencies of ERG have been reported in tumors of African American (AA) in comparison to Caucasian American (CA) men. Building on our preliminary observations, this study has focused on the increased association of the ERG-negative status with higher-grade prostate tumors in AA men. Representative whole-mount prostate sections from a matched cohort of 63 AA and 63 CA men with Gleason scores of 4+3 and those with Gleason scores of 8-10 were analyzed for ERG oncoprotein by immunohistochemistry. The striking finding of this study was that ERG expression was 3 times more likely to be present in the higher-grade index tumors of CA men compared to AA men (31 of 63 vs. 10 of 63 patients, respectively; $P < 0.0001$). Although the mechanisms underlying these differences have not been elucidated, the present study along with our previous observations underscores that ERG typing may enhance the understanding of ethnic differences and future targeted therapy of CaP.

Introduction

African American (AA) men exhibit the highest incidence and mortality from prostate cancer (CaP) compared to other

racies in the United States (1). While socioeconomic factors contribute to CaP outcomes among men of different ethnicities (2), it has also been recognized that AA men have more advanced CaP at diagnosis (3). Although there remains controversy over the role of biological differences between prostate tumors in AA and Caucasian American (CA) men, emerging data suggest the presence of differences in somatic and germline alterations (4,5).

One of the most common and validated CaP genome alterations represents fusion of the protein-coding sequences of erythroblast transformation-specific (*ETS*)-related transcription factors [predominantly *ETS*-related gene (*ERG*)] with promoter sequences of androgen-regulated genes [predominantly transmembrane protease serine 2 (*TMPS2*) gene] (6-9). The highly prevalent *ERG* fusions, present in over half of all CaPs in Western countries, result in androgen-dependent and prostate tumor-specific expression of the *ERG* fusion transcripts and a near-full-length ERG protein with a 32-amino acid deletion at the amino terminus (6-9). Evaluations of the ERG alterations at the genomic, transcriptional and protein levels have continued to suggest lower frequencies of ERG in AA CaP in comparison to CA CaP (10-13). Almost complete concordance between the detection of *ERG* gene fusions by fluorescence *in situ* hybridization and ERG protein detection by immunohistochemistry (IHC), has significantly accelerated the evaluation of the ERG protein as the surrogate of this common CaP genome alteration in pathological specimens (14-17). Studies from our and other groups indicate that the overall frequency of ERG alterations in CaP varies significantly among different ethnicities: It is highest in CA, intermediate in AA and lowest in Asian CaP patients (4,5). Our recent evaluations of representative whole-mount prostate sections from a matched cohort of 91 CA and 91 AA men demonstrated a significant difference ($P < 0.0001$) in the prevalence of the ERG oncoprotein in index tumors of CA (63%) and AA (29%) men (13). Our preliminary data also suggested that the majority of higher-grade tumors in AA patients may be ERG-negative (13). The present study focuses on comparative evaluations of ERG in higher-grade tumors in CA and AA CaP patients.

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Key words: prostate cancer, erythroblast transformation-specific-related gene, transmembrane protease serine 2 gene fusion, race, ethnicity

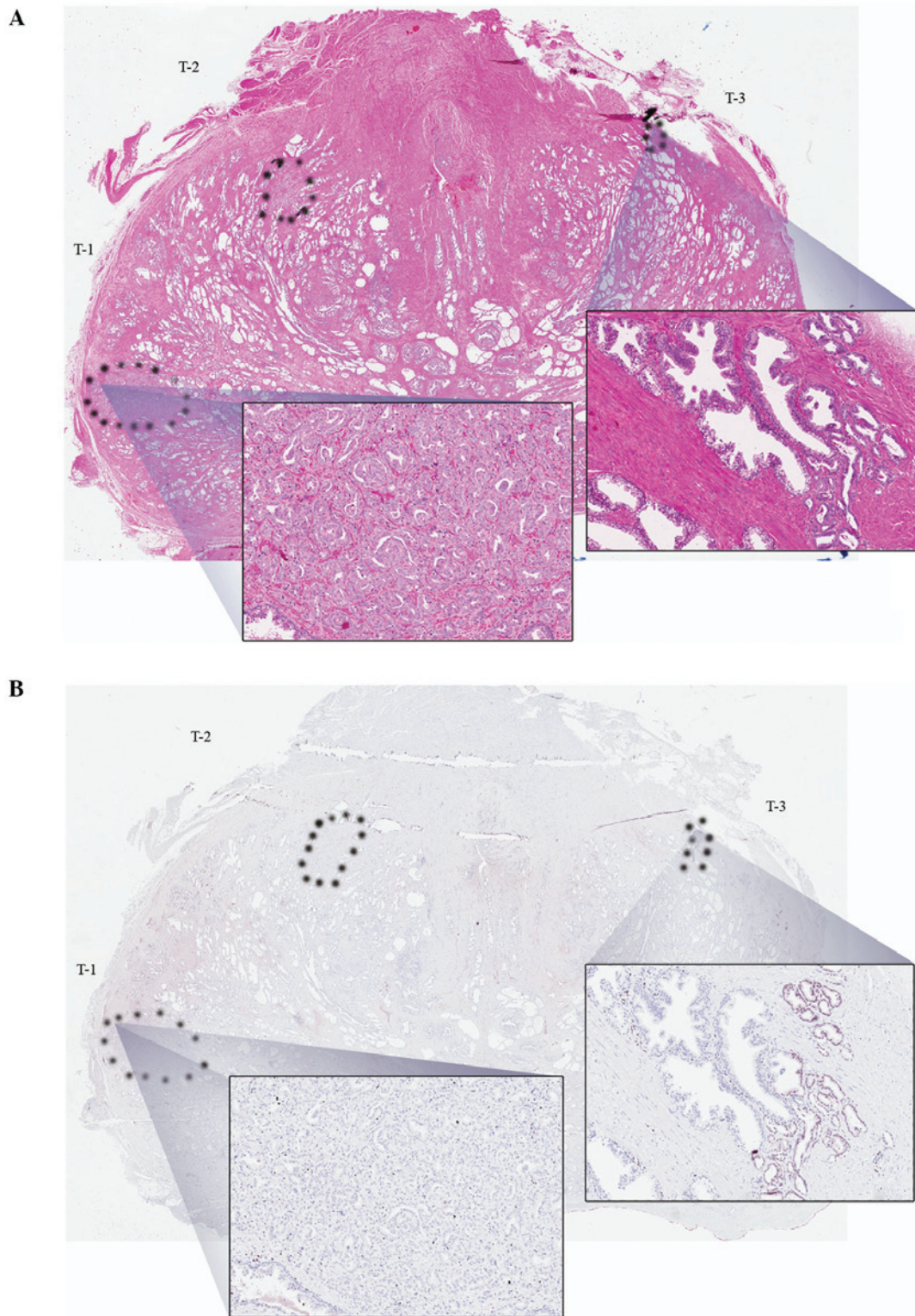


Figure 1. Representative images of whole-mount sections analyzed by hematoxylin and eosin (H&E) staining, as well as ERG immunohistochemistry (IHC), with view fields enlarged (magnification, x20). (A) H&E staining with tumor foci denoted by dotted outline. The higher power insert from T1 (index tumor) contains poorly differentiated (Gleason 4) disease. The T3 (tertiary tumor) insert on the right is well-differentiated (Gleason 3). (B) Analogous section with ERG IHC staining, in which the nuclear staining for ERG is negative in T1 and focally positive in T3.

Materials and methods

Specimens and study criteria. The Center for Prostate Disease Research database was queried to identify CaP patients who were enrolled in the Institutional Review Board-approved protocol

from Walter Reed National Military Medical Center. The CaP patients underwent radical prostatectomy (RP) between 1994 and 2011. Archived clinicopathological data were evaluated for 1,304 patients who self-identified their race. The study sample was powered for ERG evaluation. A total of 63 AA and 63 CA

Table I. Clinicopathological characteristics of all patients and breakdown across racial cohorts.

Variables	All (n=126)	AA (n=63)	CA (n=63)	P-value
Age at RP, years				0.5887
Mean (SD)	60.4 (7.1)	60.1 (7.2)	60.8 (7.1)	
PSA at diagnosis, ng/ml				0.2718
Median (range)	6.7 (0.9-5,065)	6.9 (1-5,065)	6.5 (0.9-23.4)	
Pathological T stage				0.2008
pT2	49 (38.9)	28 (44.4)	21 (33.3)	
pT3 or higher	77 (61.1)	35 (55.6)	42 (66.7)	
Gleason sum				0.8538
4+3	47 (37.3)	24 (38.1)	23 (36.5)	
8-10	79 (62.7)	39 (61.9)	40 (63.5)	
ECE				0.6855
Negative	49 (43.0)	26 (44.8)	23 (41.1)	
Positive	65 (57.0)	32 (55.2)	33 (58.9)	
SV				0.2496
Negative	91 (72.8)	48 (77.4)	43 (68.2)	
Positive	34 (27.2)	14 (22.6)	20 (31.8)	
Margin status				0.3230
Negative	83 (69.2)	44 (73.3)	39 (65.0)	
Positive	37 (30.8)	16 (26.7)	21 (35.0)	

AA, African American; CA, Caucasian American; RP, radical prostatectomy; SD, standard deviation; PSA, prostate-specific antigen; ECE, extracapsular extension; and SV, seminal vesicles invasion.

patients matched for age at RP and Gleason scores of 8-10 and 4+3 of prostate tumors met the study inclusion criteria.

IHC analyses of the ERG. Representative whole-mount 4- μ m cross-sections from each prostatectomy specimen were selected. The index tumor consisting of the largest tumor with the highest grade was identified along with all other tumor foci in each specimen. Specimens for ERG IHC were cut and stained with a highly specific anti-ERG monoclonal antibody (clone 9FY; Biocare Medical Inc., Concord, CA, USA) as previously described (13,14). The index tumor and all other tumors were classified as ERG-positive (any number of tumor cells positive) or negative (all tumor cells negative). Fig. 1 provides representative examples.

Sample size and statistical analysis. Categorical patient clinicopathological data were described across race using frequencies and percentages. Continuously measured variables were compared using measures of central tendency, namely mean, median and standard deviation. The Chi-square test was used to compare the distribution of the clinicopathological characteristics between the CA and AA cohorts, as well as IHC status (positive vs. negative) for the AA vs. CA cohorts. Biochemical recurrence (BCR), was defined as 2 consecutive prostate-specific antigen (PSA) measurements of ≥ 0.2 ng/ml at least 8 weeks post-RP. Unadjusted Kaplan-Meier estimate curves and multivariable Cox proportion hazards analysis were used to evaluate the prognostic significance of ERG oncoprotein on BCR-free survival. The log-rank test was used

to test for differences in the Kaplan-Meier curves by ERG status. $P < 0.05$ was considered to indicate a statistically significant difference. All data analyses were conducted using SAS software, version 9.3 (SAS Institute, Cary, NC, USA).

Results

Clinicopathological characteristics. The study cohort of 126 patients (63 CA and 63 AA) did not exhibit significant differences in clinicopathological variables across race (Table I). The majority of the tumors had Gleason scores of 8-10 and pT3 disease (Table I). This patient cohort provided an 80% power to detect a 25-30% absolute difference across race for ERG positivity (two-sided P -value=0.05).

ERG status by race and grade. Overall, 46% of the patients had ≥ 1 ERG-positive tumor foci. The index tumor was ERG-positive in 41 of the 126 patients. In CA men, the index tumor was ERG-positive in 31 of 63 patients (49%), which was significantly higher compared to 10 of 63 patients (16%) in AA men ($P < 0.0001$) (Table II). CA men were also significantly more likely to have any tumor focus positive for ERG compared to AA men (59 vs. 41%, $P = 0.0042$, data not shown). ERG-positive status was significantly lower in higher-grade (16%) compared to lower-grade (34%) index tumors of AA men ($P = 0.04$), which was not the case in CA men (Table II).

ERG as a predictor of recurrence. ERG was not found to be an independent predictor of BCR in this cohort (Table III).

Table II. Prevalence of ERG positivity across race in high-grade (Gleason score, 8-10 and 4+3) index tumors (upper lane, present study) and in low-grade (Gleason score, 6) index tumors (lower lane).

ERG status/grade	Total	CA	AA	P-value
ERG+/high-grade	33% (41/126)	49% (31/63)	16% (10/63)	<0.0001
ERG+/low-grade ^a	52% (35/67)	69% (24/35)	34% (11/32)	0.0051
P-value		0.0642	0.0400	

^aData obtained from Rosen *et al* (17). ERG, erythroblast transformation-specific-related gene; CA, Caucasian American; AA, African American.

Table III. Univariable and multivariable Cox proportional hazard models for the prediction of biochemical recurrence by using ERG IHC status and clinicopathological variables.

Variables	Univariable Cox models		Multivariable Cox model	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age at RP	1.011 (0.965-1.059)	0.6481		
Log PSA	1.352 (1.062-1.723)	0.0145	1.289 (0.993-1.674)	0.0564
Race/ethnicity				
CA	1			
AA	0.705 (0.371-1.340)	0.2866		
Pathological T stage				
pT2	1		1	
pT3 or higher	4.737 (1.972-11.379)	0.0005	5.749 (1.729-19.115)	0.0043
Gleason sum				
4+3	1		1	
8-10	1.858 (0.879-3.928)	0.1048	1.272 (0.545-2.968)	0.5777
SV				
Negative	1		1	
Positive	2.240 (1.183-4.241)	0.0133	1.159 (0.571-2.354)	0.6827
Margin status				
Negative	1		1	
Positive	2.276 (1.193-4.342)	0.0126	0.890 (0.427-1.855)	0.7562
ERG IHC status				
ERG-	1			
ERG+	1.366 (0.704-2.652)	0.3564		

ERG, erythroblast transformation-specific-related gene; IHC, immunohistochemistry HR, hazard ratio; CI, confidence interval; RP, radical prostatectomy; PSA, prostate-specific antigen; CA, Caucasian American; AA, African American; SV, seminal vesicles. P-values in bold print denote statistically significant differences (<0.05).

Pathological stage was an independent predictor of BCR [hazard ratio (HR)=5.749, P=0.0043] and there was a trend towards higher serum PSA levels at diagnosis (HR=1.289, P=0.0564) (Table III).

Discussion

CaP is a multifocal, heterogeneous disease with a variable clinical course. Two cancers of the same grade and stage do not necessarily exhibit similar progression characteristics and CaP does not behave equally across age groups or

ethnicities (1-5,18). Molecular alterations are likely involved in the ethnic differences of CaP and we sought to describe the prevalence of ERG in higher-grade disease in AA and CA men with a focus on index tumors. High Gleason scores are recognized as surrogates of aggressive disease and are independently predictive of BCR (19).

Studies from our and other groups have demonstrated significantly lower frequencies of ERG in CaP of AA men in comparison to that of CA men (5,12,13). Our previous preliminary observation indicated more significant differences in ERG in high-grade tumors of AA compared to those of CA men.

This adequately powered study addressed this issue by using matched cohorts of CA and AA CaP specimens. A striking finding of this study was that ERG was significantly (3 times) more likely to be present in the higher-grade index tumors of CA men compared to those of AA men (31 of 63 vs. 10 of 63 patients, respectively; $P < 0.0001$). Thus, although ERG may be the most common oncogenic alteration in CA men, it does not appear to be the case in AA men, particularly not in those with higher-grade CaP. The biological basis underlying this observation remains to be elucidated; these results nonetheless support the association of an ERG-negative status with more aggressive disease in AA men. These data also suggest that ERG may not be the primary driver of higher-grade CaP in AA men.

While there is a general agreement that *ERG* is a highly prevalent and early oncogenic alteration in CaP and it defines a large subtype of prostate tumors, it is also important to recognize that there are significant proportions of ERG-negative prostate tumors for which a common driver gene alteration is not known. Emerging data from the present and other studies underscore the higher prevalence of the ERG-negative subtype of CaP in AA and Asian men (4,5). The higher frequency of high-grade ERG-negative tumors in AA men likely reflects the presence of distinct genomic alterations associated with the initiation and progression of this subtype of CaP.

The utility of ERG detection in CaP is apparent in the diagnostic setting and ERG typing of tumors may also be of significant value for biological classification and future targeted therapy. However, the utility of ERG in assessing CaP progression remains controversial, which may be attributed to multifactorial causes, including specific patient cohort, disease stage and assay type (8,17). In this high-grade cohort, the ERG protein status was not found to be correlated with disease progression.

In summary, this study provides important observations on the predominance of ERG-negative high-grade CaP in AA men. The biological implications of these observations are far-reaching, particularly in delineating biological typing and future treatment of CaP tumors in men of different ethnicities.

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